

REMARKS

Claims 1-10 are active in the present application.

Applicants have now submitted a substitute Sequence Listing and a corresponding computer-readable Sequence Listing. The sequence information recorded in the corresponding computer-readable Sequence Listing is identical to the paper copy of the substitute Sequence Listing. Support for all of the sequences listed in the substitute Sequence Listing is found in the present application as originally filed. No new matter is believed to have been introduced by the submission of the substitute Sequence Listing and the corresponding computer-readable Sequence Listing.

Applicants submit that the present application is ready for examination on the merits. Early notice to this effect is earnestly solicited.

Respectfully submitted,

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IN THE SPECIFICATION

Page 5, please replace paragraph at lines 15-16 as follows:

(A) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing;

Page 5, please replace paragraph at lines 17-21 as follows:

(B) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

Page 5, please replace paragraph at line 24 to page 6 at line 1 as follows:

(A) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing;

Page 6, please replace paragraph at lines 2-6 as follows:

(B) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

Page 8, please replacement paragraph at lines 2-3 as follows:

(C) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing;

Page 8, please replace paragraph at lines 4-8 as follows:

(D) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing, wherein the amino acid includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

Page 8, please replace paragraph at lines 11-12 as follows:

(C) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing;

Page 8, please replace paragraph at lines 13-17 as follows:

(D) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

Page 8, please replace paragraph at lines 21-23 as follows:

(e) a DNA containing a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing.

Page 8, please replace paragraph beginning at line 24 to page 9, lines 1-4 as follows:

(f) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing or a probe prepared therefrom under a stringent condition and encoding a protein having an erythrose reductase activity.

Page 9, please replace paragraph at lines 13-15 as follows:

(g) a DNA containing a base sequence comprising at least nucleotides Nos. 408 to 1077 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing.

Page 9, please replace paragraph at lines 16-20 as follows:

(h) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 408 to 1077 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing under a stringent condition and encoding a protein having an erythrose reductase activity.

Page 10, please replace paragraph at lines 14-15 as follows:

(E) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing;

Page 10, please replace paragraph at lines 16-20 as follows:

(F) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

Page 10, please replace paragraph at lines 23-24 as follows:

(E) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing;

Page 11, please replace paragraph at lines 1-5 as follows:

(F) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

Page 11, please replace paragraph at lines 9-11 as follows:

(i) a DNA containing a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing.

Page 11, please replace paragraph at lines 12-16 as follows:

(j) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing or a probe prepared therefrom under a stringent condition and encoding a protein having an erythrose reductase activity.

Page 12, please replace paragraph at lines 1-3 as follows:

(k) a DNA containing a base sequence comprising at least nucleotides Nos. 408 to 1121 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing.

Page 12, please replace paragraph at lines 4-8 as follows:

(1) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 408 to 1121 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing under a stringent condition and encoding a protein having an erythrose reductase activity.

Page 18, please replace paragraph at lines 2-7 as follows:

For example, referring to the amino acid sequences of the aldo-keto reductase families, parts of the partially decoded amino acid (cf. SEQ. ID Nos. [6] 9 and [7] 10 in the Sequence Listing) can be selected and sense primers and anti-sense primers (cf. SEQ. ID Nos. [4] 7 and [5] 8 in the Sequence Listing) can be designed from the respective sequences.

Page 18, please replace paragraph at lines 15-20 as follows:

Using the thus-obtained single strand cDNA as a template and the sense primer (cf. SEQ. ID No. [4] 7 in the Sequence Listing) and antisense primer (cf. SEQ. ID No. [5] 8 in the Sequence Listing) designed in advance, a probe was amplified by PCR reaction. In this manner, the present inventors succeeded in obtaining a cDNA fragment having a length of 398 bp as a PCR product.

Page 21, please replace paragraph at lines 9-13 as follows:

The amino acid sequence determined based on this base sequence is also shown in SEQ. ID No. [1] 2 in the Sequence Listing. The above amino acid sequence contains a partially decoded amino acid sequence, and the protein having this amino acid sequence was the erythrose reductase type III protein.

Page 21, please replace paragraph at lines 20-24 as follows:

The amino acid sequence of the erythrose reductase type III protein (cf. SEQ. ID No. [1] 2 of the Sequence Listing) is a novel amino acid having low homology with the previously clarified amino acid sequences of human aldose reductase and of yeast (Saccharomyces cerevisiae) gcy protein.

Page 22, please replace paragraph at lines 15-18 as follows:

The present inventors have found a base sequence of a total length of 1,077 bp shown in SEQ. ID No. [2] 3 in the Sequence Listing as the DNA of a protein having erythrose reductase activity of the second aspect of the present invention.

Page 22, please replace paragraph at lines 19-23 as follows:

The amino acid sequence determined based on this base sequence is also shown in SEQ. ID No. [2] 4 in the Sequence Listing. Since the above amino acid sequence is identical with the partially decoded amino acid sequence, the protein having this amino acid sequence was the erythrose reductase type II protein.

Page 23, please replace paragraph at lines 8-12 as follows:

The amino acid sequence of the erythrose reductase type II protein (cf. SEQ. ID No. [2] 4 of the Sequence Listing) is a novel amino acid sequence having low homology in comparison with the previously clarified amino acid sequences of human aldose reductase and of yeast gcy protein.

Page 24, please replace paragraph at lines 5-8 as follows:

The present inventors have found a base sequence of a total length of 1,121 bp shown in SEQ. ID No. [3] 5 in the Sequence Listing as the DNA of a protein having erythrose reductase activity of the third aspect of the present invention.

Page 24, please replace paragraph at lines 9-13 as follows:

The amino acid sequence determined based on this base sequence is also shown in SEQ. ID No. [3] 6 in the Sequence Listing. Since the above amino acid sequence is identical with a partially decoded amino acid sequence, the protein having this amino acid sequence was the erythrose reductase type I protein.

Page 24, please replace paragraph at lines 21-24 to page 25, at line 1 as follows:

The amino acid sequence of the erythrose reductase type I protein (cf. SEQ. ID No. [3] 6 of the Sequence Listing) is a novel amino acid sequence having low homology in comparison with the previously clarified amino acid sequences of human aldose reductase and of gcy protein.

Page 25, please replace paragraph at lines 4-9 as follows:

The protein having an erythrose reductase activity according to the present invention may comprise an amino acid sequence containing one or more substitution, deletion, insertion, addition, or inversion at one or more sites with respect to the amino acid sequence of SEQ. ID No. [1] 2, [2] 4 or [3] 6 in the Sequence Listing, if erythrose reductase activity exists.

Page 25, please replace paragraph at lines 10-22 as follows:

The protein that comprises the amino acid sequence containing one or more substitution, deletion, insertion, addition or inversion at one or more sites with respect to the amino acid sequence of SEQ. ID No. [1] 2, [2] 4 or [3] 6 in the Sequence Listing as such can

be obtained, for example, by site specific mutation method (Methods in Enzymology, 100, pp. 448 (1983)), mutation treatment and in addition natural occurring mutation such as a difference in species or strain of an organism, and the like. Also, they can be obtained by manuals of experiments on genetic recombination (Nucleic Acid Res. 10, pp. 6487 (1982), Methods in Enzymol. 00, pp. 448 (1983)), PCR method (Molecular Cloning 2nd Ed., Cold Spring Harbor Laboratory Press (1989); PCR A Practical Approach IRL Press pp. 200 (1991)).

Page 27, please replace paragraph at lines 14-22 as follows:

The DNA encoding the protein having an erythrose reductase activity of the second aspect of the present invention may be not only DNA containing the base sequence of base Nos. 1 to 399, which is, out of the base sequence described in SEQ. ID No. [2] 3 in the Sequence Listing, on the N-terminal domain where it is predicted that the NAD(P)H binding site is mainly located but also DNA that hybridizes with a probe prepared from the above base sequence under stringent conditions and encodes a protein having an erythrose reductase activity.

Page 27, please replace paragraph at lines 23-24 to page 28 at lines 1-5 as follows:

Also, the DNA may be not only DNA containing the base sequence of base Nos. 408 to 1077, which is, out of the base sequence described in SEQ. ID No. [2] 3 in the Sequence Listing, a portion on the C-terminal where the erythrose or erythritol binding site may be present but also DNA that hybridizes with a probe prepared from the above base sequence under stringent conditions and encodes a protein having an erythrose reductase activity.

Page 28, please replace paragraph at lines 11-19 as follows:

The DNA encoding the protein having an erythrose reductase activity of the third aspect of the present invention may be not only DNA containing the base sequence of base

Nos. 1 to 399, which is, out of the base sequence described in SEQ. ID No. [3] 5 in the Sequence Listing, on the N-terminal domain where it is predicted that the NAD (P) H binding site is mainly located but also DNA that hybridizes with a probe prepared from the above base sequence under stringent conditions and encodes a protein having an erythrose reductase activity.

Page 28, please replace paragraph at lines 20-24 and page 29 lines 1-2 as follows:

Also, the DNA may be not only DNA containing the base sequence of base Nos. 408 to 1121, which is, out of the base sequence described in SEQ. ID No. [3] 5 in the Sequence Listing, a portion on the C-terminal where the erythrose or erythritol binding site may be present but also DNA that hybridizes with a probe prepared from the above base from 35% to 20s to recover the fractions having erythrose reductase activity.

Page 36, please replace paragraph at lines 14-21 as follows:

(3) Design of a primer used in PCR reaction

Of the partially decoded amino acid sequence, those amino acid sequences (cf. SEQ. ID Nos. [6] 2 and [7] 10 in the Sequence Listing) selected with reference to the amino acid sequences in among aldo-keto reductase family were used as a sense primer (cf. SEQ. ID No. [4] 7 in the Sequence Listing) and an antisense primer (cf. SEQ. ID No. [5] 8 in the Sequence Listing) in the PCR reaction described hereinbelow.

Page 37, please replace paragraph at lines 19-24 as follows:

Using the thus-obtained cDNA as a template, the sense primer (cf. SEQ. ID No. [4] 7 in the Sequence Listing) and antisense primer (cf. SEQ. ID No. [5] 8 in the Sequence Listing) for PCR designed in (3) above, and Pfu DNA polymerase (produced by STRATAGENE), PCR reaction was carried out 25 cycles, each cycle being 94°C, 1 minute - 40°C, 1 minute - 72°C, 1 minute.

Page 41, please replace paragraph at lines 15-18 as follows:

As a result of analyses, this revealed to be a base sequence described in SEQ. ID No. 1 in the Sequence Listing of a full length of 1,119 bp. The translation of the base sequence into amino acid was also shown in SEQ. ID No. [1] 2 in the Sequence Listing.

Page 42, please replace paragraph at lines 1-5 as follows:

The erythrose reductase type III protein consisting of the amino acid sequence described in SEQ. ID No. [1] 2 in the Sequence Listing revealed to be a novel sequence having low homology with the known amino acid sequences such as the previously elucidated human aldose reductase enzyme and yeast gcy protein.

Page 44, please replace paragraph at lines 21-24 as follows:

As a result of analyses, it was revealed that this is a base sequence described in SEQ. ID No. [2] 3 in the Sequence Listing of a full length of 1,077 bp. The translation of the base sequence into amino acid was also shown in SEQ. ID No. [2] 4 in the Sequence Listing.

Page 45, please replace paragraph at lines 9-15 as follows:

From this, it was revealed that the DNA encoding the erythrose reductase type II protein of the present invention has the sequence described in SEQ. ID No. [2] 3 so that the erythrose reductase type II gene of the present invention revealed to be a DNA encoding a polypeptide having a novel amino acid sequence.

(2) Expression of Recombinant Erythrose Reductase Type II Using E. coli

Page 45, please replace paragraph at lines 16-20 as follows:

Based on the N-terminal side of the base sequence described in SEQ. ID No. [2] 3 in the Sequence Listing except the initiation codon (atg), a primer was prepared so as to have a BamH I site. Also, based on the C-terminal side of the same base sequence, another primer was synthesized so as to have an Xho I site.

Page 46, please replace paragraph at lines 22-24 to page 47, line 1 as follows:

As a result of analyses, it was revealed that this is a base sequence described in SEQ. ID No. [3] 5 in the Sequence Listing of a full length of 1,121 bp. The translation of the base sequence into amino acid was also shown in SEQ. ID No. [3] 6 in the Sequence Listing.

Page 47, please replace paragraph at lines 2-4 as follows:

The present inventors have found a base sequence of a total length of 1,121 by shown in SEQ. ID No. [3] 5 in the Sequence Listing by the DNA sequencing.

Page 47, please replace paragraph at lines 12-18 as follows:

From this, it was revealed that the DNA encoding the erythrose reductase type I protein of the present invention has the sequence described in SEQ. ID No. [3] 5 and thus the erythrose reductase type I gene of the present invention revealed to be a DNA encoding a polypeptide having a novel amino acid sequence.

(2) Expression of Recombinant Erythrose Reductase Type I Using E. coli

Page 47, please replace paragraph at lines 19-23 as follows:

Based on the N-terminal side of the base sequence described in SEQ. ID No. [3] 5 in the Sequence Listing except the initiation codon (atg), a primer was prepared so as to have a BamH I site. Also, based on the C-terminal side of the same base sequence, another primer was synthesized so as to have an Xho I site.

IN THE CLAIMS

--1. (Amended) A protein shown in (A) or (B) below:

(A) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing;

(B) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

2. (Amended) A DNA encoding a protein shown in (A) or (B) below:

(A) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing;

(B) a protein having an amino acid sequence of SEQ. ID No. [1] 2 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

9. (Amended) A protein shown in (C) or (D) below:

(C) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing;

(D) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

10. (Amended) A DNA encoding a protein shown in (C) or (D) below:

(C) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing;

(D) a protein having an amino acid sequence of SEQ. ID No. [2] 4 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition

or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

11. (Amended) The DNA as claimed in claim 10, wherein the DNA comprises one shown in (e) or (f) below:

(e) a DNA containing a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing.

(f) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing or a probe prepared therefrom under a stringent condition and encoding a protein having an erythrose reductase activity.

13. (Amended) The DNA as claimed in claim 10, wherein the DNA comprises a DNA shown in (g) or (h) below:

(g) a DNA containing a base sequence comprising at least nucleotides Nos. 408 to 1077 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing.

(h) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 408 to 1077 out of the nucleotide sequence described in SEQ. ID No. [2] 3 in the Sequence Listing or a probe prepared therefrom under a stringent condition and encoding a protein having an erythrose reductase activity.

17. (Amended) A protein shown in (E) or (F) below:

(E) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing;

(F) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition

or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

18. (Amended) A DNA encoding a protein shown in (E) or (F) below:

(E) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing;

(F) a protein having an amino acid sequence of SEQ. ID No. [3] 6 in the Sequence Listing, wherein the amino acid sequence includes substitution, deletion, insertion, addition or inversion of one or several amino acids and wherein the protein has an erythrose reductase activity.

19. (Amended) The DNA as claimed in claim 18, wherein the DNA comprises one shown in (i) or (j) below:

(i) a DNA containing a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing.

(j) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 1 to 399 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing or a probe prepared therefrom under a stringent condition and encoding a protein having an erythrose reductase activity.

21. (Amended) The DNA as claimed in claim 18, wherein the DNA comprises a DNA shown in (k) or (l) below:

(k) a DNA containing a base sequence comprising at least nucleotides Nos. 408 to 1121 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence Listing.

(l) a DNA hybridizing with a base sequence comprising at least nucleotides Nos. 408 to 1121 out of the nucleotide sequence described in SEQ. ID No. [3] 5 in the Sequence

Listing or a probe prepared therefrom under a stringent condition and encoding a protein having an erythrose reductase activity.--



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